

CLAIMS

1. A chemical conjugate between an immunoglobulin Fab fragment and
5 molecular entities imparting diagnostic or therapeutic utility, whereby the
only sites of conjugation on the Fab fragment are one or both of the
sulfhydryl groups deriving from the selective and quantitative reduction of
the inter-chain disulfide bond of said Fab fragment and whereby said
molecular entities imparting diagnostic or therapeutic utility have at least
10 one free sulfhydryl-reactive group, characterized in that the conjugation
stoichiometric molar ratio molecular entity to Fab fragment is in the range
from 0.95 to 1.05 or in the range from 1.95 to 2.05.
2. A conjugate according to claim 1, wherein a first of said sulfhydryl
15 groups deriving from the selective and quantitative reduction of the inter-
chain disulfide bond is quantitatively functionalized by reaction with one
of said molecular entities imparting diagnostic or therapeutic utility.
3. A conjugate according to claim 2, wherein also the second one of
20 said sulfhydryl groups deriving from the selective and quantitative
reduction of the inter-chain disulfide bond is quantitatively functionalized
by reaction with a second of said molecular entities imparting diagnostic or
therapeutic utility, said second molecular entity being different or identical
to the first one.
- 25 4. A conjugate according to claim 1, wherein both of said sulfhydryl
groups deriving from the selective and quantitative reduction of the inter-

chain disulfide bond are quantitatively symmetrically functionalized by reaction with a stoichiometric excess of one of said molecular entities imparting diagnostic or therapeutic utility.

5 5. A conjugate according to claims 1 or 2, wherein one of said
sulfhydryl groups deriving from the selective and quantitative reduction of
the inter-chain disulfide bond is chemically modified by reaction with a
chemical moiety non-imparting diagnostic or therapeutic utility, said
chemical moiety being preferably selected among protective groups of the
thiol group or small alkylating or arylating agents.

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6. A conjugate according to claims 1 to 5, wherein said molecular
entities imparting diagnostic or therapeutic utility comprise derivatives of
chelating agents for, or chelates of, radionuclides, paramagnetic metal ions
or luminescent metal ions, a chromophoric fluorescent or a phosphorescent
15 molecule, a biotin molecule, a hapten recognized by a distinct antibody or
fragment thereof, an avidin or streptavidin molecule, a therapeutic drug, a
lipophilic chain bearing molecular entity incorporated into liposomes,
phospholipid-stabilized microbubbles, triglyceride- or polymer-based
microspheres, microballoons which carry the diagnostic or therapeutic
20 agent.

7. A conjugate according to claim 6, wherein said molecular entities
further comprise one or more functional groups which may be used, as
such or after deprotection or after chemical modification, as targets for the
25 selective attachment of a second Fab fragment, equal or different from the
first one, or of a second molecular entity imparting diagnostic or
therapeutic utility.

8. A conjugate according to claims 1 to 6, wherein said sulfhydryl reactive groups comprise iodoacetyl, bromoacetyl, vinyl, maleimido groups or polyfluorobenzene or dinitrofluorobenzene derivatives.

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9. A conjugate according to any one of the preceding claims, wherein the Fab fragment is a recombinant Fab.

10. A process for the preparation of the conjugates of claims 1 to 9,
10 comprising:

a) the selective and quantitative reduction of the inter-chain disulfide bond of a Fab fragment to give two free sulfhydryl groups;

b) the quantitative functionalization of one or both of the sulfhydryl groups from step a) with molecular entities having at least one free
15 sulfhydryl-reactive group and imparting diagnostic or therapeutic utility, to give mono- or diconjugate compounds, said diconjugates deriving from either symmetric or asymmetric functionalization of the sulfhydryl groups.

20 11. The process of claim 10, wherein said selective and quantitative reduction of the inter-chain disulfide bond is performed with a phosphine, preferably tributylphosphine and tris-(carboxyethyl)-phosphine.

12. The process of claim 11, wherein the reducing agent is tris-
25 (carboxyethyl)-phosphine.

13. The process of claim 11, wherein the reduction is carried out mixing

the reacting species under buffered conditions giving a final buffered aqueous reaction solution having the following characteristics:

	Fab concentration	:	1 – 100 μ M;
	Phosphine concentration	:	0.1 – 10 mM;
5	pH of the buffered solution	:	4 – 8;
	reaction time	:	5 – 180 min;
	reaction temperature	:	4 – 45 °C.

14. The process of claim 13, wherein the preferred conditions are the following:

	Fab concentration	:	1.5 – 10 μ M or 1 – 5 μ M;
	Phosphine concentration	:	0.5 – 5 mM;
	pH of the buffered solution	:	5 – 7;
	reaction time	:	25 – 70 min;
15	reaction temperature	:	25 – 40 °C.

15. The process of claim 10, wherein said quantitative functionalization of step b) is performed immediately at the end of the reduction step a), in the same reaction medium, by adding a buffered aqueous solution of the conjugating molecular entity, without purifying the reduced Fab fragment.

16. The process of claim 15, wherein the final buffered aqueous reaction solution has the following characteristics:

	Fab concentration	:	2 – 5 μ M;
25	Phosphine concentration	:	0.5 – 5 mM;
	conjugating moiety concentration	:	0.1 – 100 mM;
	pH of the buffered solution	:	5 – 7;

reaction time : ≥ 30 min;
reaction temperature : $4 - 45$ °C or $20 - 40$ °C.

17. N^2, N^2 -bis[2-[bis(carboxymethyl)amino]ethyl]- N^6 -[4-(2,5-dioxo-1H-
5 pyrrol-1-yl)-1-oxobutyl]-L-lysine as intermediate compound for the
preparation of conjugates of claim 1.

18. Pharmaceutical compositions containing as active ingredients the
conjugate compounds of claims from 1 to 9.

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19. Compositions according to claim 18, wherein said conjugate
compounds are formulated in the form of suspensions, solutions, emulsions
for parenteral administration, lyophilizates to be reconstituted before use.

15 20. Diagnostic compositions according to claim 18, wherein the dose of
the active ingredient ranges from 0.1 to 10 mg of conjugate per single
administration.

21. Therapeutic compositions according to claim 18, wherein the dose of
20 the active ingredient ranges from 10 to 500 mg of conjugate per single
administration.

22 Compositions according to claim 18, for use in analytical
immunochemical tests *in vitro*